

TEMPERATURE TOLERANCE AND OXYGEN CONSUMPTION RATES FOR JUVENILE SOUTHERN FLOUNDER *PARALICHTHYS* *LETHOSTIGMA* ACCLIMATED TO FIVE DIFFERENT TEMPERATURES

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ABSTRACT

Critical thermal maximum (CTM), lethal thermal tolerance (LT), and oxygen consumption rates ($\mu\text{g O}_2/\text{g fish}/\text{min}$) of juvenile southern flounder (average weight 5.5 g) were determined for acclimation temperatures (AT) of 13, 17, 21, 25, and 29 C. A total of 75 fish were used to measure CTM and LT in salinities of 0, 12, and 34 ppt. Three replicates of two fish each were used to measure oxygen consumption rates in 34 ppt. Salinity had a significant effect on both CTM and LT ($P < 0.05$). The mean CTM for 0 ppt was 0.46 C lower than the mean CTM for 12 ppt and 0.84 C lower than the mean for 34 ppt. The mean LT for 0 ppt was 0.40 C lower than the mean LT for 12 ppt and 0.61 C lower than the mean for 34 ppt. The LT was 20.39 C higher than acclimation temperature at 13 C but only 9.85 C higher at 29 C. The highest LT was 38.85 C for fish acclimated to 29 C. The oxygen consumption rate increased from 1.26 to 4.53 $\mu\text{g O}_2/\text{g fish}/\text{min}$ as temperature increased from 13 to 29 C. The highest Q_{10} values, for oxygen consumption, occurred between 21 C and 25 C. Between 13 C and 17 C the Q_{10} was 2.37, between 17 C and 21 C the Q_{10} was 2.50, between 21 C and 25 C the Q_{10} was 2.68, and between 25 C and 29 C the Q_{10} was 1.29. Based on the relationship between LT and preferred temperature, and the observed decline in Q_{10} for oxygen consumption, we calculated that the final preferred temperature (FP) for juvenile southern flounder is between 25 C and 29 C in salinities from 0 to 34 ppt.

INTRODUCTION

The southern flounder *Paralichthys lethostigma* has potential as a species for aquaculture (Waters 1999). Little is known about the effect of acclimation temperature on thermal tolerance and oxygen consumption, and knowledge of the thermal tolerance for juvenile southern flounder will aid in grow-out facility site selection. Likewise, oxygen consumption data will help in the development of oxygen management strategies.

Southern flounder grow well in fresh and salt water (Lasswell et al. 1977; Daniels et al. 1996). This euryhaline ability has generated interest in their potential for aquaculture. Several studies have been done to develop captive spawning techniques (Arnold et al. 1977; Henderson-Arzapalo et al. 1988; Berlinsky et al.

1996) and larviculture methods (Daniels et al. 1996; Denson and Smith 1997; Jenkins and Smith 1997; Smith et al. 1999). However, little information is available on the environmental requirements for grow-out.

The natural geographic range of the southern flounder in the wild extends from the Albemarle Sound of North Carolina to Jupiter Inlet, Florida, USA, on the Atlantic Coast and from Caloosahatchee Estuary Florida, USA, to northern Mexico in the Gulf of Mexico (Ross 1980). Adult southern flounder can be found in coastal water systems in salinities ranging from full-strength sea water to fresh water. During the months of December through January they migrate to the open ocean to spawn (Smith et al. 1975). The pelagic eggs hatch at sea and the developing larvae typically reenter the estuaries

just as they are completing metamorphosis (Burke et al. 1991).

Preferred temperature is commonly determined through the use of either a vertically or horizontally arranged temperature gradient tank (Tsuchida and Fukataki 1991). These test tanks, however, are inadequate for flounder as this species has a strong natural desire to stay hidden by not moving rather than to seek out a preferred temperature within a tank (Jun Kita personal communication, Marine Ecology Research Institute, Chiba, Japan). Recent studies by Tsuchida (1995) and Kita et al. (1996) have reported an indirect method of determining the preferred temperature of fish. Tsuchida (1995) reported a linear relationship between final preferred temperature and lethal tolerance. The final preferred temperatures of 14 marine fish species were plotted against their lethal temperatures yielding a linear relationship with an r^2 of 0.981 ($LT = 0.741FP + 17.549$). This relationship can be used to calculate the final preferred temperature of other species, given the relationship between lethal temperature and acclimation temperature ($LT = aAT + b$). This calculation is possible because the final preferred temperature is defined as the temperature where preferred temperature equals acclimation temperature. Thus, by replacing acclimation temperature with final preferred temperature, the formulas for the two equations can be combined leaving the final preferred temperature to be solved ($aFP + b = 0.741FP + 17.549$).

The final preferred temperature coincides with optimum temperature for growth (Brett 1971; Kellogg and Gift 1983). The point where the Q_{10} for oxygen consumption starts to decrease with increasing acclimation temperature also corresponds to the optimal temperature for growth (Kita et al. 1996). Thus, the final preferred temperature may be determined indirectly, based on the relationship between oxygen consumption and acclimation temperature.

The purposes of this study were 1) to determine how acclimation temperature affects thermal tolerance and oxygen consumption rates and 2) to indirectly determine the final preferred temperature for juvenile southern flounder.

MATERIALS AND METHODS

Hatchery-raised juvenile southern flounder from the Tidewater Research Station (TRS) hatchery in Plymouth, North Carolina, USA, weighing $5.5 \text{ g} \pm 1.9 \text{ g}$, were shipped to the Marine Ecology Research Institute in Onjukumachi, Chiba, Japan on 9 June 1999. Two wk prior to the experiments, juveniles were acclimated to the five test temperatures (13, 17, 21, 25 and 29 C) and fed frozen krill and moist pellets.

Thermal Tolerance

Fish at each temperature were given 3 d to acclimate to salinities of 0, 12, and 34 ppt. Fifteen thermal tolerance tests were run, one for each temperature and salinity combination. A total of 75 fish were transferred to the experimental tanks 1 h prior to the initiation of the tests. The experimental tanks were closed systems controlled by a ceramic heater and cooling device (Matsushita Electric NU-301 AHD) and a programmable thermostat (Shimaden FP21). From the acclimation temperatures, the test water was heated in increments of 5 C/h. The critical thermal maximum (CTM), where the fish lost their equilibrium, and lethal temperature (LT), where opercular movement ceased, were recorded as described by Tsuchida (1995). After the experiments, water samples were taken to confirm salinities via an inductively coupled salinometer (601 MK-III Yo-KAL Environmental Electronics).

Oxygen Consumption

Five oxygen consumption tests were run, one for each acclimation temperature at 34 ppt salinity. A total of 10 fish were transferred to the respirometry chamber (Fig. 1) approximately 20 h prior to the start of the experiment. The respirometry chamber was sunk in a water bath maintained at the desired acclimation temperature ($\pm 0.2 \text{ C}$) by a water heater and chiller (AQUA C101A-5). The flow into the respirometry chamber was cut off and the dissolved oxygen concentration was measured every 20 sec with an oxygen probe (TOA DO-25A with OE-211 oxygen electrode TOA Electronics Ltd.). Measurements continued until the oxygen

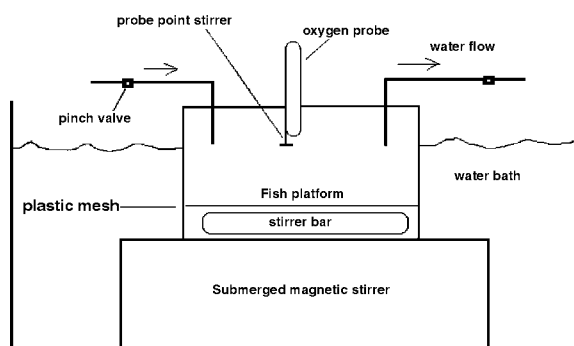


Figure 1. Respirometry Chamber used to measure oxygen consumption of southern flounder *Paralichthys lethostigma*.

concentration decreased from 100% to 80% saturation over a period ranging from 2-4 h. Oxygen consumption rate ($\mu\text{g O}_2/\text{g fish}/\text{min}$) was calculated according to Kita et al. (1996). From the oxygen consumption rates, Q_{10} was calculated as:

$$Q_{10} = (\text{Rate 1}/\text{Rate 2})^{(10/(\text{Temp 2}-\text{Temp 1}))}$$

where,

Rate 1 = the oxygen consumption rate at temperature 1

Rate 2 = the oxygen consumption rate at temperature 2

Temp 1 = the lower of the two temperatures used to determine oxygen consumption

Temp 2 = the higher of the two temperatures used to determine oxygen consumption

RESULTS

Temperature Tolerance

The salinity varied as follows: 0.20 ± 0.15 , 12.10 ± 3.43 and 34.24 ± 0.09 ppt. Salinity had a significant ($P < 0.05$) effect on both CTM and LT. The mean CTM for 0 ppt was 0.46 C lower than the mean CTM for 12 ppt and 0.84 C lower than the mean for 34 ppt. The mean LT for 0 ppt was 0.40 C lower than the mean LT for 12 ppt and 0.61 C lower than the mean for 34 ppt. The difference between the CTM at 0 ppt and the CTM at 34 ppt was greatest at 13 C. A similar trend was found for LT (Table 1). The mean LT was 20.39 C higher than acclimation temperature at 13 C but only 9.85 C higher at 29 C (Fig. 2). A similar trend was found for CTM (Fig. 3). The highest mean LT was 38.85 C for fish acclimated to 29 C.

Although salinity had a statistically significant effect on LT, the magnitude of this effect is on average less than 0.5 C. Thus, a single function across all salinities was developed to calculate the final preferred temperature ($\text{LT} = 0.336\text{AT} + 29.54$). Using the function for this line,

Table 1. Effect of salinity on critical thermal maximum (CTM) and lethal temperature (LT) at five different acclimation temperatures for juvenile southern flounder *Paralichthys lethostigma*. Values are means (\pm sd) for five fish. Means followed by a different letter are statistically different ($P < 0.05$).

Critical thermal maximum					
Temperature (C)					
Salinity (ppt)	13	17	21	25	29
0	$32.56 \pm 0.36\text{a}$	$34.78 \pm 0.08\text{a}$	$36.36 \pm 0.26\text{a}$	$37.18 \pm 0.13\text{a}$	$38.04 \pm 0.18\text{a}$
12	$32.92 \pm 0.08\text{a}$	$35.36 \pm 0.09\text{b}$	$36.72 \pm 0.18\text{b}$	$37.66 \pm 0.21\text{b}$	$38.50 \pm 0.25\text{b}$
34	$33.74 \pm 0.75\text{b}$	$36.00 \pm 0.12\text{c}$	$37.08 \pm 0.8\text{c}$	$37.78 \pm 0.05\text{b}$	$38.58 \pm 0.00\text{b}$
Lethal temperature					
Temperature (C)					
Salinity (ppt)	13	17	21	25	29
0	$32.88 \pm 0.57\text{a}$	$34.98 \pm 0.13\text{a}$	$36.80 \pm 0.14\text{a}$	$37.98 \pm 0.05\text{a}$	$38.70 \pm 0.14\text{a}$
12	$33.14 \pm 0.05\text{a}$	$35.84 \pm 0.26\text{b}$	$37.16 \pm 0.08\text{b}$	$38.12 \pm 0.05\text{b}$	$38.94 \pm 0.06\text{b}$
34	$34.08 \pm 0.80\text{b}$	$36.10 \pm 0.00\text{c}$	$37.22 \pm 0.14\text{b}$	$38.18 \pm 0.08\text{b}$	$38.96 \pm 0.06\text{b}$

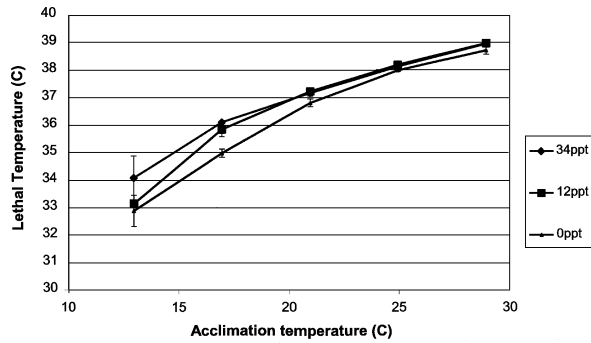


Figure 2. Lethal temperature vs. acclimation temperature for juvenile southern flounder *Paralichthys lethostigma* at three different salinities.

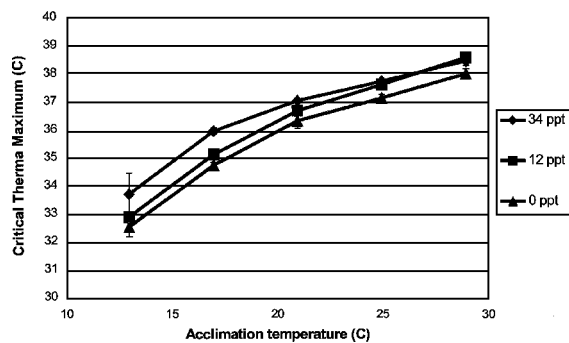


Figure 3. Critical thermal maximum vs. acclimation temperature for juvenile southern flounder *Paralichthys lethostigma* at three different salinities.

the calculated final preferred temperature is 29.61 C. This corresponds to the point where the line for lethal tolerance vs. acclimation temperature for southern flounder crosses the line for lethal temperature vs. final preferred temperature for 14 marine species (Tsuchida 1995; Fig. 4).

Oxygen Consumption

The oxygen consumption rate increased with increasing temperature and ranged from 1.26

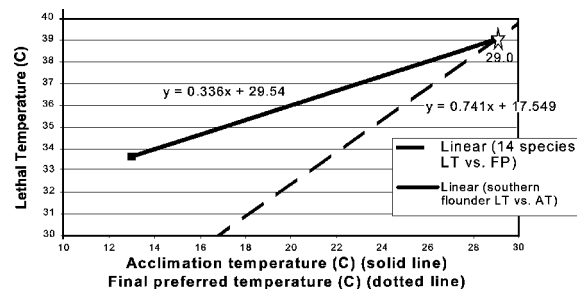


Figure 4. Lethal temperature vs. acclimation temperature for southern flounder *Paralichthys lethostigma* and lethal temperature vs. final preferred temperature as described by Tsuchida (1995).

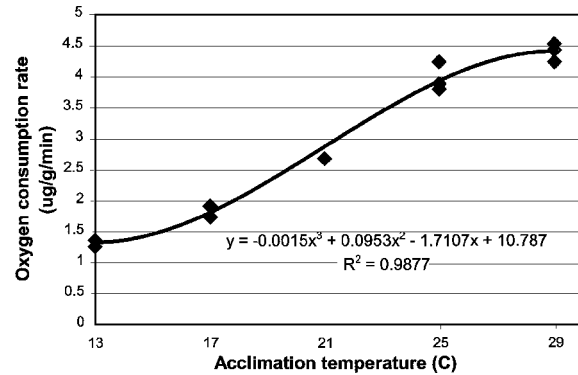


Figure 5. Juvenile southern flounder *Paralichthys lethostigma* oxygen consumption rate (μg/g/min) at five different temperatures.

to 4.53 μg O₂/g fish/min (Fig. 5). The highest Q₁₀ values occurred between 21 C and 25 C. Between 13 C and 17 C the Q₁₀ was 2.37, between 17 C and 21 C the Q₁₀ was 2.50, between 21 C and 25 C the Q₁₀ was 2.68, and between 25 C and 29 C the Q₁₀ was 1.29. Using the point where a drop in the Q₁₀ becomes apparent (Kita et al. 1996), these results suggest that the final preferred temperature of juvenile southern flounder is between 25 and 29 C.

DISCUSSION

Although salinity has a significant effect on the thermal tolerance of juvenile southern flounder, the magnitude of this effect is so small (<0.5 C) that it is of little practical significance to culturists. Furthermore, salinity has no effect on juvenile southern flounder growth and survival (Daniels and Borski 1998). Therefore, southern flounder can be considered to thrive in a wide range of salinity. This provides the following advantages to culturists: 1) flexibility in water source selection and 2) the ability to use varying salinities to combat pathogens.

Compared to the 14 species examined by Tsuchida (1995) the southern flounder has a relatively high thermal tolerance, similar to the sea bass *Lateolabrax japonicus* and the black sea bream *Acanthopagrus schlegelii*. This high tolerance is necessary for outside culture in eastern North Carolina as water temperatures can reach the low to middle 30 C range during the summer months.

Oxygen consumption rates were found to be temperature dependent for juvenile southern flounder. However, oxygen consumption rate varies with fish weight. Burke (1998) has determined the effect of weight on southern flounder oxygen consumption. By combining the relationship between temperature, weight and oxygen consumption, culturists will be able to predict the oxygen needs of their stock and thus manage oxygen input accordingly.

The preferred temperature for juvenile southern flounder, weighing $5.5 \text{ g} \pm 1.9 \text{ g}$, as calculated by the combination of the thermal tolerance tests and the oxygen consumption rate tests, falls within the range of 25-29 C. Davis (1998) found that the optimal temperature for growth of juvenile southern flounder was 25 C. This correlation, between final preferred temperature and optimum temperature, supports previous studies that show that fish prefer to live in the temperature where growth is optimized (Brett 1971; Kellogg and Gift 1983).

This study was completed with southern flounder raised from eggs collected from a single female. Additional replications of this work are needed to account for possible variations in the thermal tolerance and oxygen consumption rates that may exist within the North Carolina population and between the different strains that occur over the entire natural range.

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